EXHIBIT

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CELLULAR AND MOLECULAR IMMUNOLOGY

THIRD EDITION

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for immunization. The screening method depends on the antigen being used. For soluble antigens, the usual technique is RIA or ELISA; and for cell surface antigens, a variety of assays for antibody binding to viable cells can be used (see Laboratory Uses of Antibodies later in this chapter). Once positive wells are identified (i.e., wells containing hybridomas producing the desired antibody), the cells are cloned in semisolid agar or by limiting dilution, and clones producing the antibody are isolated by another round of screening. These cloned hybridomas produce monoclonal antibodies of a desired specificity. Hybridomas can be grown in large volumes or as ascitic tumors in syngeneic mice in order to produce large quantities of monoclonal antibodies.

Two features of this somatic cell hybridization make it extremely valuable. First, it is the best method for producing a monoclonal antibody against a known antigenic determinant. Second, it can be used to identify unknown antigens present in a mixture because each hybridoma is specific for only one antigenic determinant. For instance, if several hybridomas are produced that secrete antibodies that bind to the surface of a particular cell, each hybridoma clone will secrete an antibody specific for only one surface antigenic determinant. These monoclonal antibodies can then be used to purify different cell surface molecules, some of which may be known molecules and others that may not have been identified previously. Some of the commonest applications of hybridomas and monoclonal antibodies include the following:

(1) Identification of phenotypic markers unique to particular cell types. The basis for the modern classification of lymphocytes and mononuclear phagocytes is the binding of population-specific monoclonal antibodies. These have been used to define "clusters of differentiation" for various cell types (see Chapter 2).

(2) Immunodiagnosis. The diagnosis of many infectious and systemic diseases relies upon the detection of specific antigens and/or antibodies in the circulation or in tissues, using monoclonal antibodies in immunoassays.

(3) Tumor diagnosis and therapy. Tumor-specific monoclonal antibodies are used for detection of tumors by imaging techniques and for immunotherapy of tumors *in vivo*.

(4) Functional analysis of cell surface and secreted molecules. In immunologic research, monoclonal antibodies that bind to cell surface molecules and either stimulate or inhibit particular cellular functions are invaluable tools for defining the functions of surface molecules, including receptors for antigens. Antibodies that neutralize cytokines are routinely used for detecting the presence and functional roles of these protein hormones *in vitro* and *in vivo*.

At present, hybridomas are most often produced by fusing HAT-sensitive mouse myelomas with B cells from immunized mice, rats, or hamsters. The same principle is used to generate mouse T cell hybridomas, by fusing T cells with a HAT-sensitive, T cell–derived tumor line; uses of such monoclonal T cell populations are described in Chapter 7. Attempts are being made to generate human monoclonal antibodies, primarily for administration to patients, by developing human myeloma lines as fusion partners. (It is a general rule that the stability of hybrids is low if cells from species that are far apart in evolution are fused, and this is presumably why human B cells do not form hybridomas with mouse myeloma lines at high efficiency.) As we shall discuss later in the chapter, only small portions of the antibody molecule are responsible for binding to antigen; the remainder of the antibody molecule can be thought of as a "framework." This structural organization allows the DNA segments encoding the antigen-binding sites from a murine monoclonal antibody to be "stitched" into a complementary DNA encoding a human myeloma protein, creating a hybrid gene. When expressed, the resultant hybrid protein, which retains antigen specificity, is referred to as a "humanized antibody." Humanized antibodies offer an alternative strategy for generating monoclonal antibodies that may be safely administered to patients.

When blood or plasma forms a clot, antibodies remain in the residual fluid, called **serum.** A sample of serum that contains a large number of antibody molecules that bind to a particular antigen is commonly called an **antiserum.** (The study of antibodies and their reactions with antigens is therefore classically called **serology.**) The number of antibody molecules in a serum specific for a particular antigen is often measured by serially diluting the serum until binding can no longer be observed; sera with a large number of antibody molecules specific for a particular antigen are said to be "strong" or have a "high titer."

Plasma or serum glycoproteins are traditionally separated by solubility characteristics into albumins and globulins and may be further separated by migration in an electric field, a process called electrophoresis. Elvin Kabat and colleagues demonstrated that most antibodies are found in the third fastest migrating group of globulins, named **gamma globulins** for the third letter of the Greek alphabet. Another common name for antibody is **immunoglobulin** (Ig), referring to the immunity-conferring portion of the gamma globulin fraction. The terms immunoglobulin and antibody are used interchangeably throughout this book.

Currently, antibody molecules are generally purified from plasma or other natural fluids by a two-step procedure. The first step is to precipitate antibodies from the biologic fluid by adding a concentration of ammonium sulfate that ranges from 40 to 50 per cent of saturation. Under these conditions, albumin and most small molecules remain in solution, so that partially purified antibody can be collected in a pellet by centrifugation. The antibody-containing pellet is redissolved in buffer and then purified by various forms of chromatography (the second step). When the antibody of interest in the biologic fluid is specific for a known antigen, the antigen can be immobilized on a column matrix and used to bind the antibody, a method called *affinity chromatography*. Antibody can be recovered from the column matrix by a change in pH.

Overview of Antibody Structure

A number of the structural and functional features of antibodies were determined from the early studies of these molecules:

1. *All antibody molecules are similar in overall structure, accounting for certain common physico-*

chemical features, such as charge and solubility. These common properties may be exploited as a basis for the purification of antibody molecules from fluids such as blood. *All antibodies have a common core structure of two identical light chains (each about 24 kilodaltons [kD]) and two identical heavy chains (about 55 or 70 kD)* (Fig. 3–1). One light chain is attached to each heavy chain, and the two heavy chains are attached to each other. Both the light chains and the heavy chains contain a series of repeating, homologous units, each about 110 amino acid residues in length, which fold independently in a common globular motif, called an **immunoglobulin domain** (Fig. 3–2). All Ig domains contain two layers of β-pleated sheet with three or four strands of antiparallel polypeptide chain. Certain Ig domains, such as those comprising variable regions (see later), have an extra strand in each of the two layers. As will be discussed in Chapter 7, many other proteins of importance in the immune system contain regions that use the same folding motif and show structural relatedness to Ig amino acid sequences. All molecules that contain this motif are said to belong to the **Ig superfamily,** and all of the gene segments encoding the Ig-like domains are believed to have evolved from the same common ancestral gene (see Chapter 7, Box 7–2).

2. Despite their overall similarity, *antibody molecules can be readily divided into a small number of distinct classes and subclasses, based on minor differences in physicochemical characteristics such as size, charge, and solubility and on their behavior as antigens* (Box 3–2). The classes of antibody molecules are also called **isotypes** and in humans are named IgA, IgD, IgE, IgG, and IgM (Table 3–1). IgA and IgG isotypes can be further subdivided into closely related subclasses, or subtypes, called IgA1 and IgA2, and IgG1, IgG2, IgG3, and IgG4, respectively. In certain instances, it will be convenient to refer to studies of mouse antibody. Mice have the same general isotypes as humans, but the IgG isotype is divided into the IgG1, IgG2a, IgG2b, and IgG3 subclasses. The heavy chains of all antibody molecules of an isotype or subtype share extensive regions of amino acid sequence identity but differ from antibodies belonging to other isotypes or subtypes. Heavy chains are designated by the letter of the Greek alphabet corresponding to the overall isotype of the antibody: IgA1 contains α1 heavy chains; IgA2, α2; IgD, δ; IgE, ϵ; IgG1, γ1; IgG2, γ2; IgG3, γ3, IgG4, γ4; and

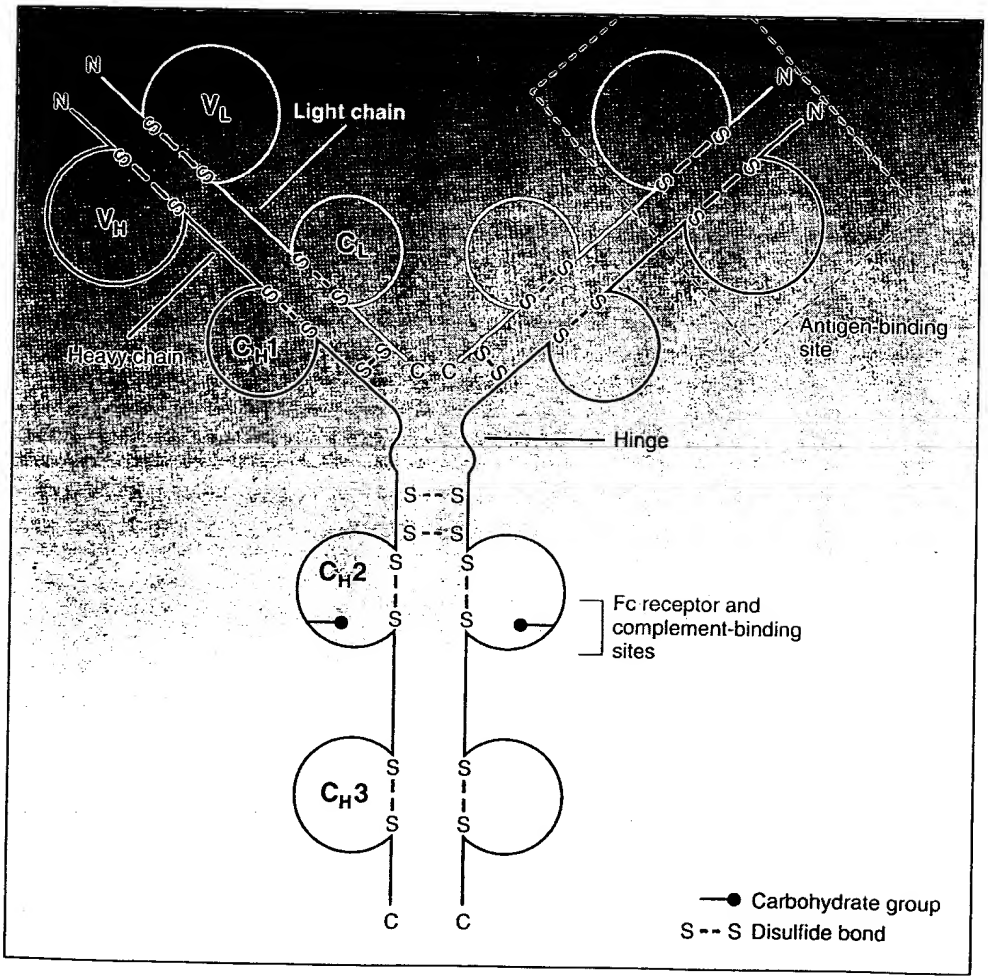

FIGURE 3–1. Schematic diagram of an immunoglobulin (Ig) molecule. In this drawing of an IgG molecule, the antigen-binding sites are formed by the juxtaposition of V_L and V_H domains. The locations of complement and Fc receptor-binding sites within the heavy chain constant regions are approximations. S--S refers to intrachain and interchain disulfide bonds; N and C refer to amino and carboxy termini of the polypeptide chains, respectively.

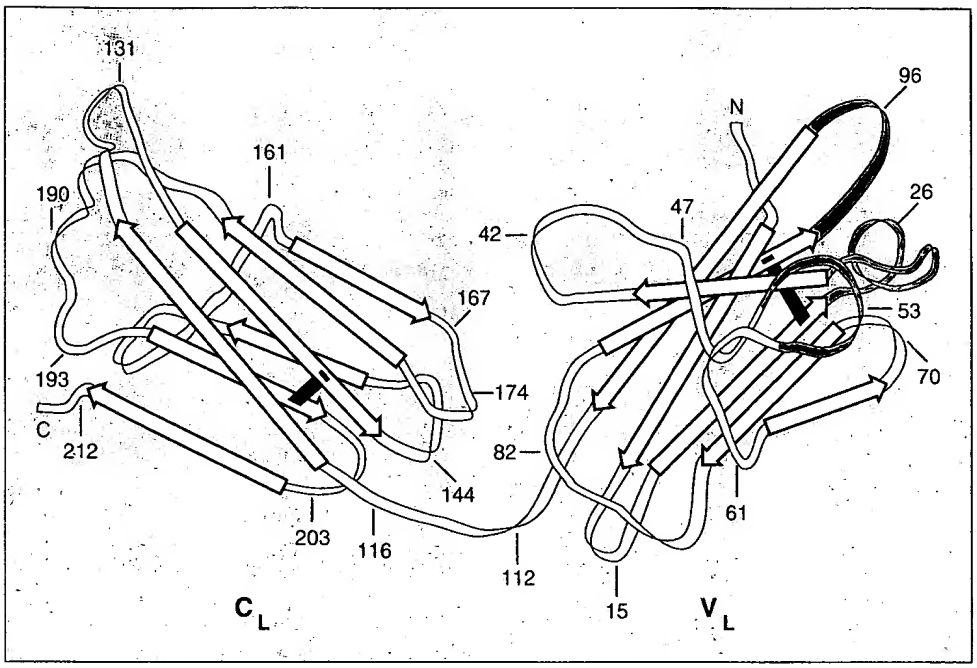

FIGURE 3-2. Polypeptide folding into immunoglobulin (Ig) domains in a human antibody light chain. The V and C regions each independently fold into Ig domains. The white arrows represent polypeptide arranged in β-pleated sheets, the dark blue bars are intrachain disulfide bonds, and the numbers indicate the positions of amino acid residues counting from the amino (N) terminus. The CDR1, CDR2, and CDR3 loops of the V region, colored in light blue, are brought together to form the antigen-binding surface of the light chain. (Adapted with permission from Edmundson, A. B., K. R. Ely, E. E. Abola, M. Schiffer, and N. Panagiotopoulos. Rotational allostery and divergent evolution of domains in immunoglobulin light chains. Biochemistry 14:3953–3961, 1975. Copyright 1975, American Chemical Society.)

IgM, μ. The shared regions of heavy chain amino acid sequences are responsible for both the common physicochemical properties and the common antigenic properties of antibodies of the same isotype. In addition, the shared regions of the heavy chains provide members of each isotype with common abilities to bind to certain cell surface receptors or to other macromolecules like complement and thereby activate particular immune effector functions. Thus, the separation of antibody molecules into isotypes and subtypes on the basis of common structural features also separates antibodies according to which set of effector functions they commonly activate. In other words, *different effector functions of antibodies are mediated by distinct isotypes and subtypes.* As we shall see later, there are two isotypes of antibody light chains, called κ and λ. The light chains do not mediate or influence the effector functions of antibodies. However, as we shall discuss shortly, both the heavy and light chains contribute to specific antigen recognition.

3. *There are more than 1×10^7, and perhaps as many as 10^9, structurally different antibody molecules in every individual, each with unique amino acid*

TABLE 3-1. Human Antibody Isotypes*

Antibody	Subtypes	H Chain (Designation)	H Chain Domains (Number)	Hinge	Tail Piece	Serum Concentration (mg/ml)	Secretory Form	Molecular Size of Secretory Form (kD)
IgA	IgA1	α1	4	Yes	Yes	3	Monomer, dimer, trimer	150, 300, or 400
	IgA2	α2	4	Yes	Yes	0.5	Monomer, dimer, trimer	150, 300, or 400
IgD	None	δ	4	Yes	Yes	Trace	—	180
IgE	None	ε	5	No	No	Trace	Monomer	190
IgG	IgG1	γ1	4	Yes	No	9	Monomer	150
	IgG2	γ2	4	Yes	No	3	Monomer	150
	IgG3	γ3	4	Yes	No	1	Monomer	150
	IgG4	γ4	4	Yes	No	0.5	Monomer	150
IgM	None	μ	5	No	Yes	1.5	Pentamer	950

* Multimeric forms of IgA and IgM are associated with J chain via the tail piece region of the heavy chain. IgA in mucus is also associated with secretory piece.

Abbreviations: Ig, immunoglobulin; kD, kilodalton.